

EDITORIAL COMMENT

Clopidogrel

Linking Evaluation of Platelet Response Variability to Mechanism of Action*

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The antiplatelet agent clopidogrel, given alone or in addition to aspirin, is effective in reducing the composite end point of death from cardiovascular causes, nonfatal myocardial infarction, or stroke in patients with acute coronary syndromes (1–3). However, many patients receiving clopidogrel still sustain these poor outcomes. Furthermore, laboratory assays of platelet response(s) to clopidogrel show wide interindividual variability, with some patients being classified as “nonresponders” or “resistant” to clopidogrel (4–9). In this issue of the *Journal*, Labarthe et al. (10) show that some patients identified as nonresponders by one assay are responders to clopidogrel by another assay. Understanding why these assays give such different results is essential to their possible future utility as guides to therapeutic decision-making and new drug development.

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The spontaneous disruption of atherosclerotic plaque or mechanical injury of an artery (as occurs during percutaneous coronary intervention) exposes the subendothelial extracellular matrix proteins von Willebrand factor and collagen to blood, leading to platelet adhesion and activation. Once activated, platelets aggregate with one another and release secondary platelet agonists, including adenosine diphosphate (ADP), that in turn recruit additional platelets to the growing thrombus. Two platelet ADP receptors, initially characterized by their pharmacologic profiles, have now been cloned and characterized: P2Y₁ (11–13), which is responsible for platelet shape change, phospholipase C activation, and calcium flux; and P2Y₁₂ (14–16), which is responsible for the inhibition of adenylyl cyclase. P2Y₁₂ has further been identified as the platelet ADP receptor targeted by thienopyridines such as clopidogrel (14). Both P2Y₁ and P2Y₁₂ are seven-transmembrane domain G-protein-coupled receptors, with P2Y₁ coupled to G_q and P2Y₁₂

coupled to G_{ai2}. Coactivation of G_q and G_i, and the signaling pathways they trigger, is necessary for full ADP-induced platelet aggregation (17).

When P2Y₁₂ function is completely eliminated, as in the case of a patient with a defective form of the P2Y₁₂ gene (18) or in platelets from P2Y₁₂-null mice (16), limited platelet aggregation still occurs unless other G_i-coupled receptors also are inhibited. However, this aggregation is rapidly reversible, demonstrating an important role for P2Y₁₂ in the stabilization of platelet aggregation. Consistent with this role in stabilizing platelet aggregation, P2Y₁₂ (unlike P2Y₁) is not desensitized within seconds after exposure to ADP but remains active over a longer period of time, thereby maintaining intracellular cyclic adenosine monophosphate at reduced levels (19,20). The functional consequences of these receptor-mediated pathways are that although both P2Y₁ and P2Y₁₂ are necessary for maximal platelet aggregation, partial inhibition of P2Y₁₂ has less of an effect on maximal aggregation and more of an effect on stabilization of aggregation. Therefore, assessing maximal platelet aggregation as an end point for functional inhibition of P2Y₁₂ by thienopyridines may not be ideal and may be further complicated by variations in the level of P2Y₁ function or in the function of receptors other than P2Y₁₂ (such as α_2 -adrenergic receptors) that are capable of triggering the G_i pathway. Indeed, polymorphisms in P2Y₁ recently have been found to be associated with increased platelet response to ADP (21) and with increased arachidonic acid-stimulated platelet aggregation in patients with a history of myocardial infarction (22).

Labarthe et al. (10) observe that inhibition of multiple end points of ADP-stimulated platelet activation by clopidogrel is more pronounced: 1) in blood anticoagulated with antithrombins (a mix of Phe-Pro-Arg-chloromethylketone [PPACK] and hirudin) rather than citrate, and 2) with an end point of late (stable) platelet aggregation rather than maximal aggregation. These results suggest that PPACK/hirudin anticoagulant and late aggregation provide a more sensitive means to test an individual's pharmacologic response to clopidogrel than previously used tests. In addition, because P2Y₁₂ and P2Y₁ play different roles in maximal aggregation versus late aggregation, the relative inhibition of these end points may provide information regarding the underlying pathogenesis in individuals with poor clinical outcomes despite treatment with clopidogrel. Accordingly, poor inhibition of maximal platelet aggregation combined with poor inhibition of late platelet aggregation may be the consequence of reduced bioavailability of clopidogrel, which may be overcome by increasing the clopidogrel dose. This possibility could be tested by guided therapy studies. Alternatively, poor inhibition of maximal aggregation, combined with significant inhibition of late aggregation, would suggest adequate inhibition of P2Y₁₂ and implicate P2Y₁ as a target for therapeutic intervention.

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The importance of the anticoagulant for testing antiplatelet drugs has been appreciated since it was observed that the glycoprotein IIb/IIIa antagonist eptifibatide is approximately two-fold less potent in blood anticoagulated with PPACK rather than citrate (23). The underlying mechanism for increased clopidogrel inhibition of platelet activation markers in blood anticoagulated without calcium chelation is unclear. Labarthe et al. (10) found that combining PPACK/hirudin anticoagulant with the late aggregation end point resulted in an almost six-fold difference in the estimation of the incidence of clopidogrel nonresponse (35% in citrate vs. 6% in PPACK/hirudin). Several mechanisms have been proposed to account for nonresponse to clopidogrel, including drug interactions (24) and genetic polymorphisms in the enzymes responsible for converting clopidogrel into its active form (25).

Because PPACK/hirudin mimics in vivo conditions by maintaining physiologic calcium levels and because late aggregation more specifically reflects the pharmacologic effects of clopidogrel, this assay (rather than maximal aggregation in citrate) may provide more relevant pharmacodynamic information on which to base treatment changes. However, pharmacodynamic efficacy is not necessarily directly proportional to clinical efficacy. Small studies have evaluated the association of clinical outcomes with several markers of clopidogrel inhibition, including the inhibition of maximal platelet aggregation in citrate (7) and vasodilator-stimulated phosphoprotein phosphorylation (4). However, the definitive answer as to which test is the best marker of clinical efficacy will require correlation of these laboratory tests with clinical outcomes in large clinical trials of patients treated with clopidogrel.

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